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(71) Applicant: **QUEST INTERNATIONAL B.V.**
1411 GP Naarden (NL)

(72) Inventors:
- **Minhas, Tony**
  Dartford, Kent DA1 2HZ (GB)
- **Casey, John**
  Wellingborough,
  Northamptonshire NN9 6PL (GB)
- **Hyliands, Della**
  Northampton NN5 5ET (GB)
- **James, Gordon**
  Northamptonshire NN10 8LG (GB)
- **Mycock, Gary**
  Northamptonshire NN10 8LH (GB)
- **Davis, Paul James**
  Felmersham Bedfordshire, MK43 7EX (GB)
- **Beggs, Thomas Stewart**
  Bedfordshire, MK44 2JX (GB)

(74) Representative: **Matthews, Heather Clare**
Keith W Nash & Co
Pearl Assurance House
90-92 Regent Street
Cambridge CB2 1DP (GB)

(54) Enzyme containing oral compositions

(57) A product for topical application in the oral cavity comprises one or more vehicles, containing in the same or separate said vehicles a polypeptide having specific binding affinity to a target site in the microflora on tooth surfaces and an enzyme functional to raise the pH in its vicinity, wherein the said enzyme is attached to said polypeptide or the product contains means to bind the enzyme to the polypeptide at least at the time of use.

In use, the enzyme, eg urease, is carried by the polypeptide, eg antibody or antibody fragment, and thereby bound to the target site at or adjacent to the dental plaque. The enzyme functions to increase pH in the immediate locality, thus inhibiting or reducing the formation of dental plaque/caries. The increase in pH also promotes breakdown of H_2S, thus inhibiting or reducing oral malodour.

EP 0 824 019 A1

Description

Field of the Invention

This invention relates to oral care, more specifically to the inhibition of dental plaque and dental caries and also the inhibition of oral malodour.

Background to the Invention

The oral microflora is a complex ecosystem which contains a wide variety of microbial species. At least one oral species, the bacterium *Streptococcus mutans*, has the ability to utilise dietary carbohydrate for the synthesis of an insoluble polysaccharide matrix which facilitates attachment to and colonisation of hard surfaces.

Dental plaque consists of this matrix polysaccharide together with oral microorganisms which exist in and on the matrix polysaccharide.

The bacterial species *Streptococcus mutans* has been identified as an important, although not the sole, contributor to dental plaque and has been shown to be capable of inducing dental caries in germ-free animals when established as a mono-infection.

Other species which occur in dental plaque as a substantial proportion of the species normally present include *S. sanguis*, and *A. naeslundii*.

Dental plaque is an acidic micro environment. The acidity is generated by oral bacteria and their extracellular enzymes which convert dietary carbohydrate into the polysaccharide matrix. The acidity is damaging to the teeth bearing the oral bacteria and plaque, but can be tolerated by the bacteria themselves. The extracellular enzymes display greater activity under acidic conditions than in a neutral or alkaline environment.

There have been a number of proposals for delivering a biologically active agent, such as a cytotoxic enzyme, to a target site by means of an antibody having specific binding The use of an antibody to attach a cytotoxic agent to a target site in the mouth has already been proposed. For example WO-A-89/11866 (Rama Biolink) discloses conjugates of bactericide and anti-*S.mutans*.

The delivery of enzymes as cytotoxic agents has been proposed. For example, Okuda et al, Infection and Immunity 27 690 (1980), describe use of two enzymes, xanthine oxidase and lactoperoxidase, chemically conjugated to antibodies against target cells. Over a period of ninety minutes, in vitro, they achieve a reduction of live candida albicans cells between one and two orders of magnitude better than that achieved with lactoperoxidase in solution.

Other disclosures of the use of antibodies or antibody fragments to target a cytotoxic agent to cells include WO90/03185 (Ideon) and WO91/00112 (Brunswick).

EP-A-450800, EP-A-451972, EP-A-453097 and EP-A-479600 (all Unilever) disclose the use of antibodies and antibody fragments to attach co-operating cytotoxic enzymes to a target site, especially oral bacteria.

Summary of the Invention

According to the present invention a product for topical application in the oral cavity comprises one or more vehicles which contain, in the same vehicle or distributed between a plurality of vehicles:

(i) a polypeptide having specific binding affinity to a target site in the microflora on tooth surfaces, and
(ii) an enzyme functional to raise the pH in its vicinity,

wherein the said enzyme is attached to said polypeptide or the product contains means to bind the enzyme to the polypeptide at least at the time of use.

By application of a product of this invention in the mouth, the enzyme is carried by the polypeptide and thereby bound to the target site at or adjacent to the dental plaque. The enzyme functions to increase pH in the immediate locality. Such reduction of acidity through operation of the enzyme is directly beneficial to the teeth bearing the plaque because it is acidity which leads to degradation of tooth surfaces and the eventual formation of caries.

There is also a less direct benefit, in that the reduction in acidity also reduces the activity of the extracellular enzymes which are responsible for creating the acidity.

Furthermore, the creation of a less acidic microenvironment may reduce the competitive advantage of the acid-tolerant oral bacteria, and so favour colonisation of tooth surfaces by bacteria which are less harmful to the teeth.

In another aspect this invention provides the use, for inhibiting or reducing the formation of dental plaque/caries by topical application in the mouth, of one or more vehicles containing a polypeptide and an enzyme as set forth above.

The invention also provides a method of inhibiting or reducing formation of dental plaque/caries comprising applying topically in the mouth one or more vehicles which are acceptable for use therein, and which contain

(i) a polypeptide having specific binding affinity to a target site in the microflora on tooth surfaces,
(ii) an enzyme functional to generate an alkaline product and thereby raise pH, said polypeptide and said enzyme being attached together or said vehicles or vehicles including means for attaching the enzyme to the polypeptide, at least at the time of use.

An important factor in oral malodour, or bad breath, is H_2S, which is generated by organisms including *Streptococci*, such as *S. sanguis*. The volatility of H_2S is pH dependent, with H_2S dissociating to hydrogen ion and the soluble and highly reactive HS^- ion with a pK_a of about 7. At pH 8.0 over 90% of H_2S is dissociated. The action in use of the enzyme of the product of the invention in raising pH therefore also promotes dissociation of H_2S in the oral cavity, so inhibiting or reducing oral malodour.

The present invention thus also provides a method of inhibiting or reducing oral malodour comprising applying topically in the mouth one or more vehicles which are acceptable for use therein, and which contain

(i) a polypeptide having specific binding affinity to a target site in the microflora on tooth sufaces,
(ii) an enzyme functional to generate an alkaline product and thereby raise pH, said polypeptide and said enzyme being attached together or said vehicles or vehicles including means for attaching the enzyme to the polypeptide, at least at the time of use.

In another aspect, the invention covers use, as agents to inhibit or reduce oral malodour, of

(i) an enzyme functional to generate an alkaline product and thereby raise pH,
(ii) a polypeptide having specific binding affinity to a target site in the microflora on tooth surfaces, and serving also for attaching the enzyme, at least at the time of use.

It is envisaged that the enzyme will generally function to generate an alkaline product, and/or convert an alkaline substrate into a product which is more alkaline, thus increasing pH in its immediate locality. Alternatively, the enzyme may generate a precursor material which then undergoes a subsequent conversion *in situ* to an alkaline product. Such further conversion might be effected spontaneously by bacteria naturally present in plaque. Enhancing the concentration of the precursor would then enhance the concentration of the alkaline product produced spontaneously from it.

The polypeptide with specific binding affinity to the target site may be an entire protein, or it may be a rather smaller polypeptide. It will usually be an antibody to the target site, or a fragment of an antibody which possesses the specific binding characteristics of an antibody.

Binding of a polypeptide to a target site entails ligand-receptor type interactions between the polypeptide and the material which is bound to it. There is an equilibrium between bound and unbound material, which can be represented by the chemical equation:

$$A + S \rightleftarrows AS$$

where A represents unbound polypeptide, S represents unbound target sites and AS represents the complex of polypeptide and target bound together. The concentrations are related by:

$$\frac{[AS]}{[A]\,[S]} = K_a$$

where K_a is a constant, referred to as the affinity constant. Specific binding affinity between a polypeptide and a target will frequently have a value of K_a of 10^6 litre mole^{-1} or greater. It may well have a value of at least 10^7 or even at least 10^8 litre mole^{-1} for example in the range 10^7 to 10^9 litre mole^{-1} which is typical of the binding affinity between an antibody and an antigen. However, even higher affinities, exceeding 10^9 litre mole^{-1} are possible and may be utilised within the scope of this invention.

This invention can be implemented using, as the binding polypeptide, polyclonal antibodies obtained by the traditional route of immunising a host animal with the target antigen (e.g. an oral bacterial species).

The invention can also utilise monoclonal antibodies, which can be derived by standard techniques from β-lyphocytes which make a polyclonal antibody response.

A further possibility for use as the binding polypeptide is an antibody fragment. Fragments which are used may be an Fab or Fv fragment, or the variable region of a heavy chain (a Dab fragment) or of a light chain. Some antibody fragments, notably Fab fragments, can be obtained by enzymic digestion of whole antibodies. Preferred routes to antibody fragments are through recombinant DNA technology, so that the fragment is expressed by a genetically trans-

formed organism. These techniques may commence with DNA or mRNA from a hybridoma which produces monoclonal antibody, but other methods use immunoglobulin V-region gene libraries from immunised or non-immunised animals.

Antibody fragments produced by recombinant DNA technology need not be identical to fragments of antibodies produced in other ways. For instance they may include sequences of amino acids which differ from those found in antibodies produced in other ways, especially sequences at the ends of the fragments. Somewhat analogously, antibody fragments produced through recombinant DNA technology may include extra amino acid sequences at their termini which have no counterpart in antibodies produced in other ways.

Techniques for the production of antibody fragments are well known in the literature. Relevant disclosures include:

Saiki et al Science 230 1350-54 (1985);
Orlandi et al PNAS USA 86 3833-7 (1989);
WO 89/9825 (Celltech);
EP-A-368684 (Medical Research Council);
WO 91/8482 (Unilever).

Through use of recombinant DNA technology it is possible to produce polypeptides which incorporate fragments of more than one antibody, and consequently have specific binding affinity to more than one antigen. Such constructs may be used in the present invention.

A possibility is that a polypeptide binding agent for use in this invention is a synthetic polypeptide which mimics the specific binding activity of a natural antibody's complementarily determining region(s). Such a polypeptide is de *facto* a fragment of an artificial antibody.

An enzyme which is particularly envisaged for use in this invention is urease. This enzyme converts urea to ammonium hydroxide and carbon dioxide. The enzyme occurs in a number of bacterial, yeast and plant species. Urease from various sources is available from Sigma Chemical Company, PO Box 14508, St. Louis, MO 63178, USA and Sigma Chemical Ltd. Poole, Dorset, England. Species envisaged as sources of urease include *Bacillus pasteurii, Methophilus* and *Helicobacter pylori.*

Another enzyme which could be used is asparaginase, more completely designated as L-asparagine amidinohydrolase which converts L-asparagine into L-aspartic acid and ammonia, so that there is an overall increase in alkalinity. It has International Union of Biochemistry number 3.5.1.1 and is also available from Sigma.

Yet another possible enzyme is arginase, more specifically L-arginine amidinohydrolase, International Union of Biochemistry number 3.5.3.1. It is also available from Sigma and converts arginine to ornithine (which is more alkaline) and urea.

As mentioned above the enzyme may alternatively function to produce an intermediate which is not itself alkaline, but undergoes further reaction *in situ* to generate an alkaline product.

Binding of the enzyme to the polypeptide which serves to bind the enzyme to the target site may be accomplished in various ways. The binding may be direct conjugation by covalent chemical bonding to an antibody or antibody fragment which exhibits specific binding affinity for an oral microorganism.

Techniques for chemical conjugation of one polypeptide to another are known. Generally these use a pair of coupling agents which are reacted separately with respective polypeptides and then reacted with each other, linking the peptides together, when they are mixed.

An example of the conjugation of polypeptides is found in Knowles et al J. Clinical Investigation, *52* 1443, (1973) which used a modification of the technique of Dutton et al, Biochem Biophys. Res. Commun. *23* 730 (1966). Another example is provided in Lal et al, Journal of Immunological Methods *79* 307 (1985).

Chemical conjugation to an antibody fragment is likely to employ an Fab fragment or an Fv fragment which has been expressed with an additional "tail" as an extension of one of its peptide chains.

Another possibility is to conjugate the enzyme to an antibody or antibody fragment which does not bind directly to a species of the oral microflora, but instead binds to another antibody or fragment which will in turn bind to the target protein. This approach utilises chemical conjugation but does not require the antibody or fragment which binds the target protein to undergo a chemical reaction which might adversely affect it.

A further possibility is for the enzyme and an antibody fragment which binds to an oral microorganism to be formed as separate parts of a single polypeptide which is expressed as a fusion protein by a genetically transformed microorganism.

Alternatives to direct chemical bonding, relying on antibody-antigen binding, are also possible and a number of such techniques are described in our published European applications EP-A-450800, EP-A-453097, EP-A-451972 and EP-A-479600 mentioned above, the disclosures of which are incorporated herein by reference. In particular EP-A-453097 describes the linking of an antibody with specific binding affinity for a target site and an antibody with specific binding affinity for an enzyme by use of a third antibody which forms a bridge between them. A similar arrangement may also be achieved using antibody fragments as described in that European publication and also our EP-A-479600.

Another way to rely on an antibody-antigen type binding is to utilise a polypeptide which incorporates two antibody fragments as mentioned earlier. One of the fragments binds to the target site while the other binds to the enzyme. Suitable is that both fragments are Fv fragments.

WO 91/08482 referred to above describes the production of a polypeptide containing two V_H chains with different specific binding affinities. WO 94/09131 describes the production of constructs containing two Fv antibody fragments with different specific binding affinities.

Holliger et al, Proc. Nat. Acad. Sci. USA 90 6444 (1993) also described the production of polypeptides which each incorporate two Fv fragments.

In this invention, the target site may be one of the bacterial species which occur in dental plaque. The effect of targeting to one such species will be to raise pH both in the vicinity of organisms of that species and also in the surrounding areas of the plaque where other bacterial species may also occur. The matrix polysaccharide of dental plaque could itself be used as the target against which the antibody or antibody fragment displays specific binding affinity.

A product of this invention comprises one or more vehicles to contain the polypeptide (e.g. antibody or antibody fragment) with binding affinity to the target and also the enzyme whose function is to raise pH.

When the enzyme is directly attached to the binding polypeptide, as a fusion protein or conjugate, this can be contained in a single vehicle. However, if the enzyme is to be attached to an antibody or antibody fragment through antibody-antigen binding involving at least one further antibody or fragment, then it may well be desirable that the enzyme and antibodies or antibody fragments are distributed among a plurality of vehicles, so that the complex of enzyme and antibodies will form only when the vehicles are mixed, for example at the time of use. In this latter case, parts of the complex may link together beforehand if they are capable of doing so and are in the same vehicle but formation of the full complex will take place only when all of its components come together. Delaying full complex formation until the time of use can be advantageous in avoiding loss of activity during storage.

Complexes of enzyme and polypeptide which are large in size tend to be unstable, and to precipitate during storage. They also diffuse more slowly. For these reasons if whole antibodies are used it can be advantageous to distribute the enzyme and antibody(ies) between different vehicles, especially if the antibodies are polyclonal, since polyclonal antibodies tend to form larger complexes than monoclonal antibodies.

Antibody fragments have the advantage of being smaller, and form smaller complexes which would be expected to retain their activity during storage better than complexes formed with whole antibodies.

Plainly, for this invention, the vehicle or vehicles which are used should be suitable to enter the mouth. There are a number of possibilities.

The simplest possibility is a sterile aqueous solution for use as a mouthwash. This could be a one component mouthwash containing the binding polypeptide chemically conjugated to the enzyme. Alternatively, when the enzyme is not directly attached to the binding polypeptides, it could be a multicomponent mouthwash consisting of a plurality of solutions containing between them

(i) antibody or antibody fragment with specific binding affinity to a site in dental plaque,
(ii) the enzyme, e.g. urease,
(iii) antibody or antibody fragment with specific binding affinity to urease,
(iv) antibody or bivalent antibody fragment with specific binding affinity to the other antibodies or antibody fragments and which is able to a form bridge between them.

There could for example be one solution containing (i) and (iii) and a second solution containing (ii) and (iv).

These individual aqueous solutions or suspensions would be mixed together by the user before use as a mouthwash, allowing formation of a complex which in the case of whole antibodies would consist of anti-target antibody: bridging antibody: anti-enzyme antibody: enzyme, as described in our EP-A453097.

Other possible forms of vehicle are a toothpaste, a lozenge which will dissolve in the mouth, chewing gum and dental floss. These forms of product could be used even when a plurality of vehicles are needed. A toothpaste with two components stored separately in a toothpaste container so that they are kept separate until the toothpaste is extruded from the container is a concept known *per se*. A lozenge to be sucked in the mouth could have various vehicles contained in separate regions of the lozenge so that the vehicles mix as the lozenge is sucked. Two different vehicle forms could be used in combination as a way to provide a plurality of vehicles: for example a toothpaste whose use is followed by a mouthwash or a lozenge.

The product may include a substrate for the enzyme or alternatively it may rely on the enzyme substrate being present at the target site.

For urease, the substrate is urea. Saliva contains a certain amount of urea, and we have found that this *in vivo* amount is a sufficiently high concentration to give changes of pH whose effect has been demonstrated *in vitro*.

A development of this invention is for the product to incorporate another enzyme with a beneficial effect which is

also delivered by means of a polypeptide with specific binding affinity to a target site in the microflora on tooth surfaces, this further enzyme being suitable for giving its beneficial function in the alkaline environment created by the first enzyme. An enzyme which is particularly envisaged as the second enzyme is urate oxidase which functions to convert uric acid to carbon dioxide and allantoin at the same time generating hydrogen peroxide. Hydrogen peroxide is rapidly decomposed in vivo, but when the enzyme which generates it is located close to cells which it is desired to attack, then the hydrogen peroxide is able to give a cytotoxic action.

Example 1

Monoclonal mouse antibodies with specific binding affinity to *S. sanguis* were produced by known techniques.

A urease-antibody conjugate was purchased from Sigma Chemical Ltd. It was urease chemically conjugated to a polyclonal goat anti-mouse antibody. The solution of this conjugate as supplied was diluted by varying amounts, as shown below, using sterile aqueous saline solution as diluent.

S. sanguis cells were suspended in sterile saline solution containing 5% w/v glucose and 0.1 % w/v urea so as to provide a concentration of approximately 10^9 cells ml^{-1}.

One sample of this suspension was used as a control. To another sample was added the urease conjugate at a dilution of 1/100.

To further samples were added the urease conjugate at the same or greater dilution, together with the mouse anti-S. *sanguis* antibody at a concentration of approximately 5µg/ml.

This acted as a bridging antibody which allowed the urease conjugate to bind to the S. *sanguis* cells. The complex which was formed consisted of the urease conjugate binding to the mouse antibody which in turn bound to the *S. sanguis* cells.

The pH of the medium was observed after periods of ½, 1, 2, 3 and 24 hours using a pH meter. The results are set out in Table 1.

Table 1

	Observed pH of medium					
Elapsed Time (hrs)	0	1/2	1	2	3	24
Control (*S. sanguis* only)	6.8	6.6	6.1	5.8	5.3	5.0
S. sanguis + conjugate 1/100 dilution	6.8	6.8	6.8	6.8	6.8	7.5
S. sanguis + conjugate 1/100 dilution + mouse anti *S. sanguis*	7.2	8.4	8.7	8.7	8.7	8.7
S. sanguis + conjugate 1/500 dilution + mouse anti *S. sanguis*	6.8	7.2	7.7	8.1	8.3	8.7
S. sanguis + conjugate 1/1000 dilution + mouse anti *S. sanguis*	6.8	6.9	7.1	7.3	7.4	7.5

As can be seen from these results, the *S. sanguis* on its own progressively made the medium acidic. Incorporation of the urease conjugate prevented this.

The pH remained stable over 3 hours and slowly thereafter became alkaline. Incorporation of the anti-*S. sanguis* antibody, so that the enzyme attached to the cells, led to alkaline pH much more quickly, even when the urease concentration was reduced.

In vivo in the mouth, it could be predicted from these results that urease conjugate which was not attached to cells or other target would be washed away by the saliva flow and have little effect, whereas urease which became attached to cells of the oral microflora would be retained in the mouth and be able to reduce or overcome the production of acidity.

Example 2

Experiments were carried out *in vitro* to demonstrate the effect of treatment with urease and antibodies on H_2S levels in the headspace above established *S. sanguis* biomass as a model of effects in the oral cavity of use of products in accordance with the invention.

Below pH 4.0, H_2S exists exclusively in the headspace. However, as the medium becomes more basic, H_2S dissociates to the soluble and highly reactive HS^- ion ($H_2S \rightleftarrows H^+ + HS^-$, $pK_a \sim 7$). In fact, at pH 8.0, > 90% of H_2S is dissociated and dissolved in the medium. Above pH 10.0, HS^- is further dissociated to S^{2-}. Significantly, in the presence of metal salts, H_2S dissolution is essentially irreversible as the highly reactive HS^- ion forms insoluble sulphide precipitates. In a preliminary study, the partial irreversible disappearance of H_2S from the headspace in the presence of metal or ammonium salts was demonstrated experimentally (data not shown).

Established *S. sanguis* biomasses were resuspended in medium (amino acid-free except cysteine) as specified below, after being treated as outlined below.

The medium composition used for the growth of oral microorganisms was as follows:

Basal medium (g/l): KNO_3 (0.1); KH_2PO_4 (1.0); K_2HPO_4 (1.5); NaCl (0.1); $(NH_4)_2PO_4$ (2.0).

Vitamins (mg/l): nicotinic acid (1.0); nicotinamide (1.0); NADH (1.0); folic acid (1.0); calcium pantothenate (1.0); riboflavin (1.0); thiamine-HCl (1.0); spermine tetrahydrochloride (1.0); spermine trihydrochloride (1.0); putrescine dihydrochloride (1.0); pimelic acid (0.1); D,L-mevalonic acid lactone (0.1); D-mevalonic acid lactone (0.1); biotin (0.1); *p*-aminobenzoic acid (0.1); lipoic acid (0.1); *B*-alanine (10.0); myo-inositol (10.0); choline chloride (50.0).

Pyridoxal species (mg/l): pyridoxal (1.0); pyridoxal-HCl (1.0); pyridoxamine dihydrochloride (1.0).

Purines/pyrimidines (mg/l): adenine (10.0); guanine (10.0); cytosine (10.0); thymine (10.0); xanthine (10.0); hypoxanthine (10.0); uracil (10.0).

Salts (mg/l): KI (4.0); $CuSO_4$ (1.0); H_3BO_3 (0.4); $FeSO_4$ (5.0); $MnSO_4$ (50.0); Na_2MoO_4 (5.0).

Other additions: hemin (5.0 mg/l); glucose (4.0g/l); $CaCl_2$(10.0 mg/l); $MgSO_4$ (0.7g/l); $NaHCO_3$ (1.0g/l)

Amino acids (mg/l): cysteine (500).

The first antibody (1st Ab) employed in the study was a murine anti-*S. sanguis* IgG monoclonal used at a final concentration of 0.1 mg/ml (in blocking buffer). The first antibody binds to bacterial cells. The second antibody (2nd Ab) was a commerically available goat anti-mouse IgG preparation conjugated to Jack Bean urease (Sigma), which was diluted by a factor of 50 (in blocking buffer) prior to use. The second antibody binds to the first antibody and catalyses urea hydrolysis. The blocking buffer (TBS-Tween) contained 29 mM Tris-HCl pH 6.8, 0.15 M NaCl, 12.5 µM EDTA and 0.5% Tween 20. The blocking buffer prevents non-specific binding and is also employed to wash away unbound antibody.

Prior to resuspension in medium, each cell pellet was exposed to one of the following treatments (all at 30°C with agitation):

(i) untreated control;
(ii) 1 h incubation in TBS-Tween;
(iii) 1 h incubation in 1st Ab, followed by 2 x 15 min washes in TBS-Tween;
(iv) 1 h incubation in 2nd Ab, followed by 2 x 15 min washes in TBS-Tween;
(v) 1 h incubation in 1st Ab, 2 x 15 min washes in TBS-Tween, 1 h incubation in 2nd Ab, 2 x 15 min washes in TBS-Tween.

Headspace VSC levels were measured after 22 h and, after 23 h, the cells were harvested and resuspended in 10mM urea/12.5 µM EDTA (15 min incubation, with agitation, at 30°C). At this point, headspace VSC levels were again measured and the pH values of the suspensions recorded. Results are given in Table 2.

Table 2

Treatment(s)	H_2S @ t = 22h (ng/ml)	H_2S @ t= 23.25h (ng/ml)	pH @ t = 23.25h
None	218.6	120.7	5.4
TBS-Tween	357.4	160.3	5.3
1st Ab	293.5	151.1	5.4
2nd Ab	403.5	202.9	5.7
1st Ab + 2nd Ab	272.9	2.3	8.5

The data presented in Table 2 clearly demonstrate that microbially-generated H_2S is removed from the headspace due to a pH increase effected by the urease-catalysed production of ammonia from urea. The effect is clearly dependent on antibody targeting as no significant pH shift (or H_2S removal) was observed when the urease-conjugated 2nd antibody was added on its own. Likewise, neither the 1st Ab nor the blocking buffer had any effect individually. Significantly, the effect is also long-lasting, an important prerequisite for use as an oral deodorant, as H_2S removal was observed > 23 h after exposure of the cells to the antibodies.

A further requirement of active ingredients to control oral maladour is that they are capable of functioning after a short contact time. Consequently, a second experiment was undertaken to determine whether the antibody-targetted urease system could remove headspace H_2S after only 5 min exposure to the *S. sanguis* cells.

For this experiment, established biomasses were resuspended as before in medium (amino acid-free except cysteine) after being treated as outlined below. The 1st and 2nd (urease-conjugated) Ab's were used at the same levels as outlined previously.

Prior to resuspension, treatments were as follows:

(i) 10 min incubation in TBS-Tween
(ii) 5 min incubation in 1st Ab, 5 min wash in TBS-Tween;
(iii) 5 min incubation in 2nd Ab, 5 min wash in TBS-Tween;
(iv) 5 min incubation in 1st Ab + 2nd Ab (mixture), 5 min wash in TBS-Tween.

Headspace VSC levels were measured after 18 h and, after 19 h, the cells were harvested and resuspended in 10 mM urea/12.5 μM EDTA (10 min incubation, with agitation, at 30°C). At this point, headspace VSC levels were again measured and the pH values of the suspensions recorded. After 20 h, the cells were pelleted and resuspended for a second time in urea/EDTA. On this occasion, the suspensions were degassed/overgassed with OFN an supplemented with chemically-generated H_2S (~ 2000 ng/vial) before being incubated for 10 min as before. At this point, VSC and pH values were again recorded. Results are given in Table 3.

Table 3

Treatment(s)	H_2S @ t=18h, ng/ml	H_2S @ t=19.2h, ng/ml (pH)	H_2S @ t=20.2h, ng/ml (pH)
TBS-Tween	213.0	76.4 (pH 5.3)	141.3 (pH 5.5)
1st Ab	226.0	86.5 (pH 5.3)	137.9 (pH 5.4)
2nd Ab	207.4	70.7 (pH 8.3)	131.7 (pH 5.9)
1st Ab + 2nd Ab	212.0	2.8 (pH 8.3)	1.1 (pH 8.2)

The data presented in Table 3 demonstrate that the antibody-targeted urease system is capable of removing headspace H_2S after only 5 min exposure to *S.sanguis* cells. Furthermore, the activity of the system was maintained after the addition of fresh medium and chemically-generated H_2S 20 h after initial exposure to the antibodies, confirming its continued effectiveness over long periods of time despite the diluting out of residual active material. The dependency of the system on antibody targeting was also confirmed.

The results of this study, as given in Tables 2 and 3, clearly demonstrate the potential of using antibody-targeted urease actives to prevent (or reverse) the volatility of microbially-produced H_2S in the oral cavity. Their viability as oral malodour counteractants is demonstrated by the fast, targeted, effective and long-lasting response they ellicit. *In situ,* it is envisaged that these actives may effect localised pH increases which could control volatile H_2S generation by *S. sanguis* and by other oral bacteria against which antibodies could also be raised.

Claims

1. A product for topical application in the oral cavity comprising one or more vehicles, containing in the same or separate said vehicles a polypeptide having specific binding affinity to a target site in the microflora on tooth surfaces and an enzyme functional to raise the pH in its vicinity, wherein the said enzyme is attached to said polypeptide or the product contains means to bind the enzyme to the polypeptide at least at the time of use.

2. A product according to claim 1, wherein the polypeptide has specific binding affinity to an oral bacterial species.

3. A product according to claim 1 or 2, wherein the polypeptide is an antibody or an antibody fragment.

4. A product according to claim 3, wherein the polypeptide incorporates fragments of two antibodies, one fragment having specific binding affinity to the said target site and the other fragment having specific binding affinity to the enzyme.

5. A product according to claim 4, wherein the fragments are both Fv fragments.

6. A product according to claim 3, wherein the said vehicle or vehicles include a second antibody or antibody fragment with specific binding affinity to the enzyme and a third antibody or antibody fragment able to bind to both the first said antibody or antibody fragment and to the second said antibody or antibody fragment.

7. A product according to any one of claims 1 to 3, wherein the enzyme is directly attached to the polypeptide.

8. A product according to claim 7, wherein the enzyme and the polypeptide with specific binding affinity are separate parts of a fusion protein.

9. A product according to any one of claims 1 to 3, wherein the enzyme is directly attached to a second polypeptide having specific binding affinity for the first said polypeptide.

10. A product according to any one of the preceding claims, wherein the vehicle or vehicles also incorporate an enzyme functional to generate hydrogen peroxide in an alkaline environment, said enzyme being attached to a polypeptide with specific binding affinity to a target site in the microflora on tooth surfaces or the product including means for attaching said second enzyme to such a polypeptide at least at the time of use.

11. A method of inhibiting or reducing formation of dental plaque/caries comprising applying topically in the mouth one or more vehicles which are acceptable for use therein, and which contain

(i) a polypeptide having specific binding affinity to a target site in the microflora on tooth surfaces,
(ii) an enzyme functional to generate an alkaline product and thereby raise pH, said polypeptide and said enzyme being attached together or said vehicles or vehicles including means for attaching the enzyme to the polypeptide, at least at the time of use.

12. Use, as agents to inhibit or reduce formation of dental plaque/caries, of (i) an enzyme functional to generate an alkaline product and thereby raise pH, (ii) a polypeptide having specific binding affinity to a target site in the microflora on tooth surfaces, and serving also for attaching the enzyme, at least at the time of use.

13. A method of inhibiting or reducing oral malodour comprising applying topically in the mouth one or more vehicles which are acceptable for use therein, and which contain

(i) a polypeptide having specific binding affinity to a target site in the microflora on tooth surfaces,
(ii) an enzyme functional to generate an alkaline product and thereby raise pH, said polypeptide and said enzyme being attached together or said vehicles or vehicles including means for attaching the enzyme to the polypeptide, at least at the time of use.

14. Use, as agents to inhibit or reduce oral malodour, of

(i) an enzyme functional to generate an alkaline product and thereby raise pH,
(ii) a polypeptide having specific binding affinity to a target site in the microflora on tooth surfaces, and serving also for attaching the enzyme, at least at the time of use.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 97 30 5852

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y,D	EP 0 479 600 A (UNILEVER PLC ET AL) * claims 1-14 * * page 4, column 5, line 12 - line 39 * * page 5, column 7, line 24 - column 8, line 2 * * page 7 *	1-14	A61K7/16 A61K7/28 A61K47/48
Y	GB 673 670 A (J. B. ROERIG AND CO) * the whole document *	1-14	
Y	US 4 269 822 A (M. A. PELLICO ET AL) * the whole document *	1-14	
A	EP 0 380 084 A (THE BIOMEMBRANE INSTITUTE) * claims 1-17 * * page 9, line 3B - line 51 *	1-14	
A	EP 0 140 49B A (LION CORPORATION) * claims 1-6,12-15 *	1-14	
A	GB 2 047 091 A (H. R. MUHLEMANN) * page 1, line 5 - line 65 *	1-14	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	17 November 1997	Siatou, E

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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E : earlier patent document, but published on, or after the filing date
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